

Vector Surveillance to Determine Species Composition and Occurrence of *Trypanosoma cruzi* Infection at Three Military Installations in San Antonio, Texas

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ABSTRACT

Chagas disease, also known as American trypanosomiasis, is caused by the hemoflagellate protozoan *Trypanosoma cruzi* which is transmitted by blood-sucking triatomine bugs (Hemiptera: Reduviidae; Triatominae). The disease is endemic to south Texas, but exists almost exclusively as a zoonosis. Chagas disease has proven to be a serious public health threat to military working dogs. In 2007, seroprevalence of Chagas disease in military working dogs in San Antonio, Texas, reached 8%. A faunal survey was conducted at 3 San Antonio area military installations (Camp Bullis, Fort Sam Houston, and Lackland Air Force Base). A total of 140 triatomines representing 4 species (*Triatoma gerstaeckeri*, *T. sanguisuga*, *T. lectularia*, and *T. indictiva*) were collected. *Trypanosoma cruzi* infected bugs were only collected at Lackland Air Force Base, where the overall infection rate was 16%. The wood excavation technique developed during this study collected all life stages. Only 2 life stages (adult and 5th instar) were positive for *T. cruzi*.

INTRODUCTION

Chagas disease or American trypanosomiasis is caused by the hemoflagellate protozoan *Trypanosoma cruzi* Chagas, which was identified by the Brazilian physician Carlos R. J. Chagas in 1909.¹ It is a serious disease that mainly occurs in Latin America (Mexico, Central and South America).² Currently, over 7 million people are infected with more than 108 million people at risk annually.² Of those infected, 21,000 are predicted to die each year.² *Trypanosoma cruzi* is mainly transmitted to humans via infected feces of hematophagous triatomine (Hemiptera: Reduviidae; Triatominae) bugs. Human transmission primarily occurs when an infected bug defecates on a host during or shortly after taking a blood-meal. Reacting to an itching sensation, the bite victim rubs fecal material into the bite site, which ultimately facilitates the transmission of the parasite. Triatomine bugs feed on many mammalian and avian hosts. They typically feed on sleeping humans at night, attacking wherever skin is exposed, but especially on the face. For this reason, the triatomine bug is often referred to as the “kissing bug.” Although the pathogen can also be transmitted to humans by blood transfusion,³ congenitally,⁴ by oral ingestion,⁵ and by organ transplantations,^{6,7} more

than 80% of all human cases are caused by vector-borne transmission.⁸

In the United States, Chagas disease exists almost exclusively in animals.⁹ Only 5 autochthonous insect-borne cases in humans have been reported in the southwestern United States.^{10,11} The first autochthonous insect-borne case of Chagas disease in the United States was reported by Woody and Woody in 1955.¹¹ Trypanosomes were discovered in the blood of a 10-month-old girl from Corpus Christi, Texas.¹¹ One of the suggested reasons for the low incidence of human Chagas disease cases in the southwestern United States is differences in the resting and blood feeding (sylvatic and peridomestic) habitats of the insect vectors.¹²

Although the reported incidence of human Chagas disease in the United States is relatively low, North American strains of *T. cruzi* have been proven to be virulent among various mammals exposed to triatomine-triatomine species in nature.¹² Some of the common mammalian species naturally infected with *T. cruzi* are raccoon (*Procyon lotor* (Linnaeus)), wood rat (*Neotoma micropus* Baird), armadillo (*Dasypus novemcinctus*

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(Linnaeus)), and domestic dog (*Canis familiaris* (Linnaeus)).^{13,14} Many dog breeds have been infected and suffered mortality because of this infestation.¹⁴ Twelve species of triatomines are known to occur in the southern half of the United States.⁹ Seven of the triatomine species including: *Triatoma gerstaeckeri* (Stal), *T. indicativa* Neiva, *T. lecticularia* (Stal), *T. protracta* (Uhler), *T. rubida* (Uhler), and *T. neotomae* Neiva, *T. sanguisuga* (Leconte) have been routinely collected from Texas.¹⁴ The overall infection rate in Texas has been found to be higher than in any other state where *T. cruzi* has been reported.¹² The *T. cruzi* infection rate among triatomine vectors in Texas has been reported as more than 50%.¹⁵

Chagas disease has proven to be a significant veterinary health threat to the military working dogs (MWDs). In late 2006, US Army veterinarians assigned to the Military Working Dog Center Veterinary Services, Lackland Air Force Base, San Antonio, TX, began to observe an increase of Chagas disease cases in the MWD population. Prior to this, only a few sporadic cases were identified through serology and clinical diagnosis. In 2007, veterinarians began to see an increase in the number of Chagas cases diagnosed. A cross-sectional serological study was conducted at one of the kennel facilities. It was demonstrated that a seroprevalence rate of 8% (24) of the dogs were positive for *T. cruzi* antibodies (S. Baty, written communication, June 30, 2007). This number was comparable to the number reported for stray dogs tested along the US border with Mexico.⁹ In 2009, several MWDs were returned to the Military Working Dog Center with reported clinical symptoms of cardiac issues further diagnosed as Chagas disease. These dogs were supporting various units deployed to the Iraq theater of operations. As a result of evacuating the MWDs, these units were left without explosive detection dogs, thus leaving the units more vulnerable to attacks using improvised explosive devices. These findings have recently brought a new sense of urgency to better understand the Chagas disease vector. The purpose of this field study was to (1) survey *Triatoma* spp. found on Lackland Air Force Base, Camp Bullis, Medina Annex, and Fort Sam Houston; (2) develop *Triatoma* collection techniques; (3) characterize *Triatoma* habitats; and (4) determine relative incidence of *T. cruzi* in field collected specimens.

MATERIALS AND METHODS

Study Area

Five sites were selected based on proximity to potential host and habitat characteristics as indicated in the literature^{9,12,13,16} (Figures 1 and 2).

Site 1. Medina Woods, N29° 23' 07.97" W98° 40' 55.76" (Figure 1). The Medina Training area consists of

woodland habitat (oak, mesquite forest and open range land) located at the northeastern edge of the Medina Annex (Figure 2A).

Site 2. The Medina Kennel, N29° 23' 13.71" W98° 39' 57.46" (Figure 1). The Medina Kennel is a fixed site facility that houses approximately 800 dogs (Figure 3A).

Site 3. Transportation Security Administration (TSA) puppy breeding program kennels (N29° 24' 06.96" W98° 37' 07.02") are located on the northeastern side of Lackland Air Force Base (Figure 1). It is located south of Wilford Hall Medical Treatment Facility and is proximate to a wooded drainage area. Both kennel sites have several floodlights operating during the night.

Site 4. Western side of Camp Bullis, N29° 40' 24.45" W98° 36' 23.74" (Figure 1). Camp Bullis is located in the Texas Hill country. Predominate vegetation consists of oak, juniper, mesquite, prickly pear cactus, yucca plants, and open range (Figure 2B).

Site 5. Fort Sam Houston, N29° 27' 38.28" W98° 25' 26.70" (Figure 1). The site is located on the northeastern side of the installation between the golf course and the horse stables.

Field Collection Techniques

Triatomine bugs were collected from April to August 2011 at the study sites using 2 techniques.

- Excavation of dead wood. The wood excavation technique involved identifying likely triatomine harborage such as a sheltered position near a potential host nesting/resting location. A visual search was conducted at the harborage site. Sites examined included hollow or rotten logs, cactus plants, dead yucca plants, and other debris piles near burrows (Figure 2).
- Dog kennels surveillance. Dog kennels were inspected daily by kennel personnel and kissing bugs (Figure 3B) were collected using forceps and placed into 50 ml collection tubes with holes drilled in the tube cap for aeration.

Rearing, Preparation of Fecal Pools, and Species Identification

Specimens were maintained in the insectary by methods described by Durvasula and Taneja.¹⁷ Insects were blood fed in vitro using a glass blood feeder¹⁸ (Figure 4A) and defibrinated rabbit blood (less than 7 days old) (Lampire, Pipersville, PA). The blood was kept at 37°C using Super RMT LAUDA hot water circulator (LAUDA Brinkman, Delran, NJ). Each blood fed insect was

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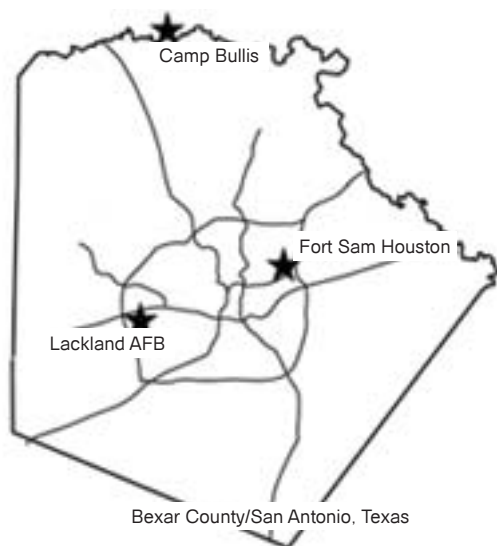
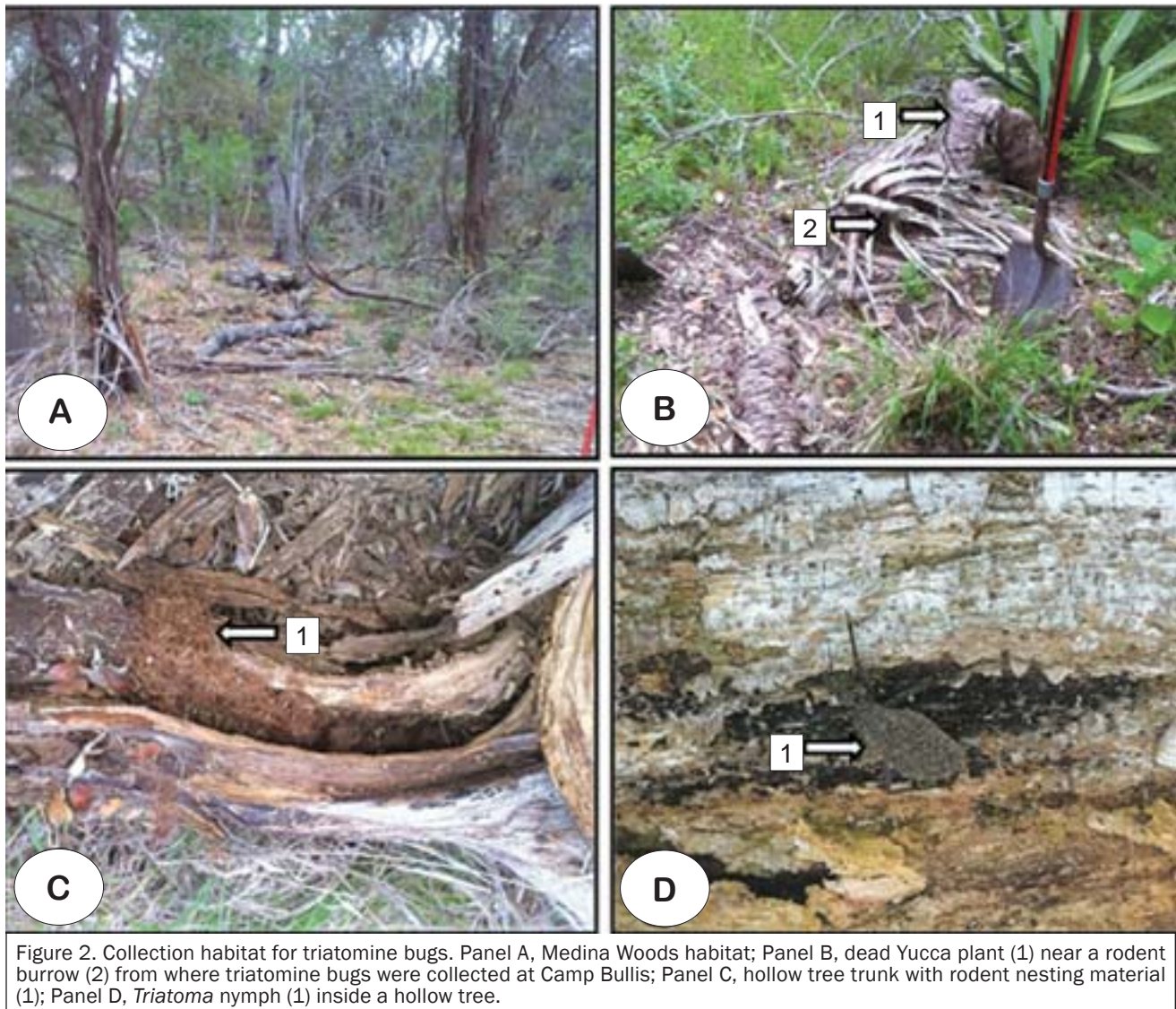


Figure 1. Map and aerial imagery of the 3 military installations in and around San Antonio, Texas, where specimens of triatomine bugs were collected, April through August 2011.





isolated individually in a 25 cm² cell culture flask, with canted neck and 0.2 µm vent cap (Corning Flask, Lowell, MA) (Figure 4B). The flasks were kept humid in plastic boxes with wet paper towels. Triatomines were identified using taxonomic keys by Lent and Wygodzinsky¹⁹ (Figure 5). A database was maintained for all the specimens brought into the laboratory.

Detection of *T. cruzi* Infection by Traditional Polymerase Chain Reaction

Sample collection. Fecal matter from triatomine bugs was collected by plastic shaft with Dacron tip wetted with M4RT media liquid (Remel, Lexena, KS). Dacron tips were placed in 2.5 mL microtubes with 500 µL aliquoted liquid media and subsequently frozen at -20°C until testing.

Sample processing. Aliquots used were subjected to one freeze/thaw cycle. From each sample, 140 µL was aliquoted for nucleic acid extraction. Specimen extractions were performed using the Qiagen QiaAmp Viral RNA Mini Kit (Qiagen Inc, Valencia, CA) according to manufacturer's minispin extraction recommendation. Feces from a subsample of the collected bugs (uninfected/infected) were checked for the presence and absence of live *T. cruzi* by magnification (×400) using a compound microscope ((Figure 4C, D) Table 1).

Primers. Primers used for amplification by traditional PCR of *T. cruzi* were:

primer set TCZ1/TCZ2:

forward primer TCZ1, 5'-CGAGCTCTTGCCCACACGGGTGCT-3'

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reverse primer TCZ2, 5'-CCTCCAAGCAGCGGATAG TTCAGG-3')²⁰

primer set S36/S36: forward primer S35, 5'-AAATAA TGTACGGGKAGATGCATGA-3'

reverse primer S36, 5'-GGGTTTCGATTGGGGTTGGT GT-3').^{2,21}

DNA amplification. The PCR was performed in 25 µl reaction mixtures consisting of 7.45 µl PCR grade water (Roche Diagnostics, Mannheim, Germany), 5 µl 5x Q-Solution (Qiagen, Valencia, CA), 5 µl GoTaq Flexi (Promega, Madison, WI), 1.5 mol MgCl₂, 0.5 µl deoxynucleoside triphosphates, and 0.15 µl of each primer (Integrated DNA Technologies, Coralville, IA). Reaction conditions (iCycler BioRad, Hercules, CA), for TCZ1/TCZ2 primers: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of amplification at 94°C for

20 seconds, 57°C for 10 seconds, and 72°C for 30 seconds, a final extension at 72°C for 7 minutes, then final hold at 4°C. Conditions for the S35 and S36 primers were processed under the following conditions: initial denaturation at 95°C for 10 minutes, followed by 35 cycles of amplification at 95°C for 30 seconds, 58°C for 30 seconds, and 72°C for one minute, then a final extension at 72°C for 10 minutes, and held at 4°C until analysis.

The completed DNA reactions were analyzed by gel electrophoresis made with 2% agarose (Fischer Scientific, Fair Lawn, NJ) in 1X Tris-Borate-EDTA Buffer (Sigma-Aldrich, St Louis, MO) solution. Each sample set was run with a negative (without DNA) and positive control (with *T. cruzi* strain). Positive controls were acquired from the Department of Defense Food and Animal Diagnostics Laboratories at Fort Sam Houston, TX

Table 1. Distribution of *Trypanosoma cruzi* infection in different species of triatomine bugs collected at 3 US military installations in San Antonio, Texas, April to August 2011.

Location	Species	PCR Results	No. Tested	Percentage Infected	Visual Validation
Camp Bullis	<i>T. gerstaeckeri</i>	Not Infected	21	-	3
	<i>T. indictiva</i>	Not Infected	11	-	1
	<i>T. lecticularia</i>	Not Infected	1	-	
	<i>T. sanguisuga</i>	Not Infected	10	-	1
	Totals		43		5
Ft. Sam Houston	<i>T. gerstaeckeri</i>	Not Infected	2	-	1
	Totals		2		1
Lackland Air Force Base					
Medina Woods	<i>T. gerstaeckeri</i>	Not Infected	11	-	3
	<i>T. gerstaeckeri</i>	Infected	2	15.4	1
	<i>T. indictiva</i>	Not Infected	1	-	
	<i>T. sanguisuga</i>	Not Infected	22	-	2
	<i>T. sanguisuga</i>	Infected	3	12.0	1
	Totals		39		7
Medina Kennel	<i>T. gerstaeckeri</i>	Not Infected	9	-	4
	<i>T. gerstaeckeri</i>	Infected	10	52.6	10
	<i>T. lecticularia</i>	Not Infected	1	-	
	<i>T. sanguisuga</i>	Not Infected	4	-	
	Totals		24		14
TSA Puppy Kennel	<i>T. gerstaeckeri</i>	Not Infected	2	-	2
	<i>T. gerstaeckeri</i>	Infected	3	60.0	3
	Totals		5		5
Totals of tested specimens			113		31
TSA indicates US Transportation Security Administration.					

RESULTS

A total of 140 triatomine-triatomine bugs representing 4 species (*T. gerstaeckeri*, *T. sanguisuga*, *T. lectularia*, and *T. indictiva*) were collected during this study (Table 2). The most prevalent triatomines collected were *T. gerstaeckeri* (49%) and *T. sanguisuga* (39%). *Triatoma gerstaeckeri* was collected at all 5 study sites, while *T. sanguisuga* was only collected at 3 study sites. The majority of *T. gerstaeckeri* were collected at Camp Bullis (37%) and Medina Kennel (35%). All life stages were collected for *T. gerstaeckeri* and *T. sanguisuga*. Four life stages (adult, 5th, 4th, and 3rd instars) were collected for *T. indictiva* and only adult collections resulted for *T. lectularia*. Only adults were collected at both kennel sites. Woodland collections from Camp Bullis and Medina resulted in collections of all life stages for *T. gerstaeckeri*.

Of 113 specimens tested using PCR for *T. cruzi*, 16% were positive. *Triatoma gerstaeckeri* (25%) and *T. sanguisuga* (8%) were the only species that tested positive for *T. cruzi*. Infected bugs were only collected at 3 (Medina Woods, Medina Kennel, and TSA) of the 5 collection sites. The TSA kennel site had the highest occurrence (60%) of infection. However, only 5 bugs were collected. Medina kennel was the site with the second highest occurrence (53%) of infection. Only 2 life stages (adult and 5th instar) were positive for *T. cruzi*.



Table 2. Distribution of species composition of triatomine bugs collected at 3 US military installations in San Antonio, Texas, April to August 2011.

Location	Species	Number	Percentage
Camp Bullis	<i>T. gerstaeckeri</i>	26	49.0
	<i>T. indictiva</i>	12	22.6
	<i>T. lecticularia</i>	1	1.9
	<i>T. sanguisuga</i>	14	26.4
	Total	53	
Ft. Sam Houston	<i>T. gerstaeckeri</i>	2	100
	Total	2	
Lackland Air Force Base			
Medina Woods	<i>T. gerstaeckeri</i>	13	27.1
	<i>T. indictiva</i>	1	2.1
	<i>T. sanguisuga</i>	34	70.8
	Total	48	
Medina Kennel	<i>T. gerstaeckeri</i>	25	78.1
	<i>T. lecticularia</i>	1	3.1
	<i>T. sanguisuga</i>	6	18.8
	Total	32	
TSA Puppy Kennel	<i>T. gerstaeckeri</i>	5	100
	Total	5	
Total Collected		140	
TSA indicates US Transportation Security Administration.			

COMMENT

This study provided an updated status on the distribution and infection prevalence of triatomine species on 3 military installations in San Antonio, TX. Pippin¹² conducted the last comprehensive Chagas disease survey of this kind in the 1960s. From 1965 to 1966, Pippin conducted an extensive Chagas disease study on Lackland Air Force base. He collected over 386 specimens using 2 collection methods: wood rat dwelling inspection and black light trapping.¹² Pippin inspected 142 wood rat dwellings and collected 229 triatomines, of which the majority were *T. sanguisuga* and *T. gerstaeckeri* nymphs. Of the specimens collected from the wood rat dwellings, 30% were infected with *T. cruzi* like organisms. While *T. sanguisuga* nymphs were the most prevalent specimens collected, *T. gerstaeckeri* nymphs had a higher infection rate for *T. cruzi*.¹²

The triatomine distribution in our study was similar to Pippin's results. However, infection rates were different. This difference was most likely due to variance of collection techniques. While Pippin concentrated on wood

rat dwellings, the majority of our specimens were collected inside the dog kennels (Figure 3). This collection technique appeared to be biased for adult specimens. Immature kissing bugs were never observed during collection at the kennel sites. Based on observed triatomine activity in the wood line near the kennels, we believe that adult kissing bugs flew to the kennels to feed. Furthermore, environmental conditions (short grass, roads between wood lines, and kennel daily cleaning regimen with high pressure water) at the kennel are not conducive for the establishment of triatomine colonies. Our results are similar to recent studies conducted in other areas of Texas.^{9,12-15} In a study conducted by Kjos et al,¹⁴ higher infection rates were observed in dog kennels in a domestic setting when compared to sylvan settings.

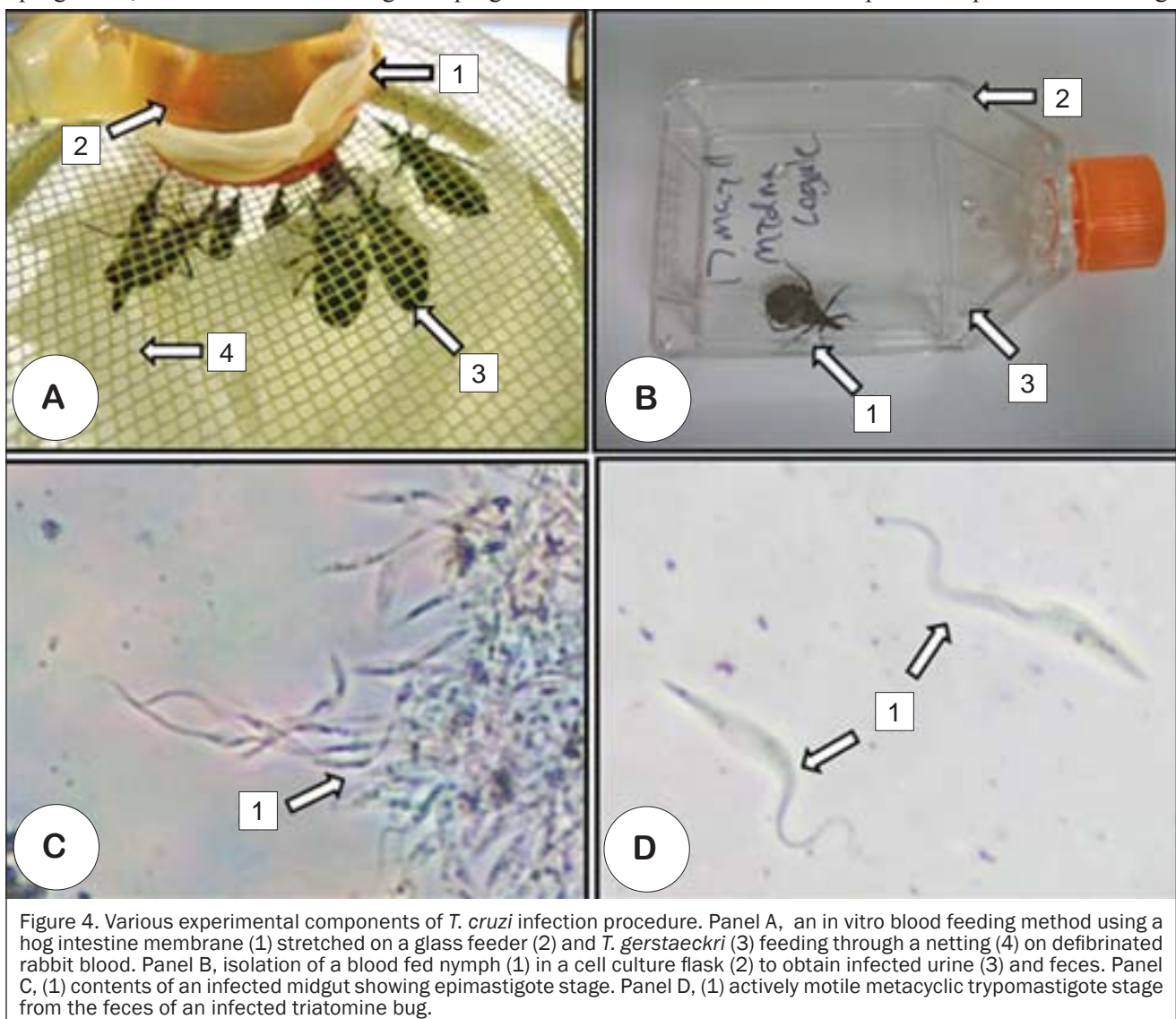
Our results indicate that the area with the highest *T. cruzi* infection rate is in and around the military working dog kennels. This is consistent with clinical observations at the MWD Center. Dogs observed at the MWD Center have exhibited multiple clinical signs. Canines affected with Chagas disease develop either acute or

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chronic disease. Generally, dogs develop signs characterized by right-sided heart failure and cardiac arrhythmias.²² Acute myocarditis, such as sudden collapse and death of a previously normal dog was observed in one case. Other symptoms associated with acute cardiac issues include lethargy, pale mucous membranes with slow capillary refill time, weak pulse, tachyarrhythmias, and respiratory arrest. Additionally, other clinical symptoms in dogs that do not die suddenly will exhibit a generalized lymphadenopathy, diarrhea, weight loss due to anorexia, fever, hepatosplenomegaly caused by the right-sided heart failure, ascites, and some neurological signs characterized by pelvic limb ataxia.²³ Dogs which overcome the acute symptoms and become chronic survivors will develop further cardiac issues to include arrhythmias which can be exacerbated by exercise or hard work on the battlefield. As the chronic myocarditis progresses, the heart muscle undergoes a progressive

cardiac degeneration and dilation. The result is a bilateral enlarged heart with flaccid thin walls that exhibit abnormal electrical impulses and arrhythmias on an electrocardiograph, along with possible respiratory distress. These cases can often be confused with chronic dilative cardiomyopathy observed in other large breeds of dogs.

Effective surveillance for Chagas disease vectors is essential for the development of a control program. Few studies have been published regarding the ecology of North American Triatomines. Most research efforts have been conducted in endemic countries (Mexico, Guatemala, Brazil, and Peru) in Central and South America. Surveillance techniques in these areas yielded large numbers of triatomines. This is mainly due to the difference in abundance, behavior, and biology. The main South American species responsible for Chagas



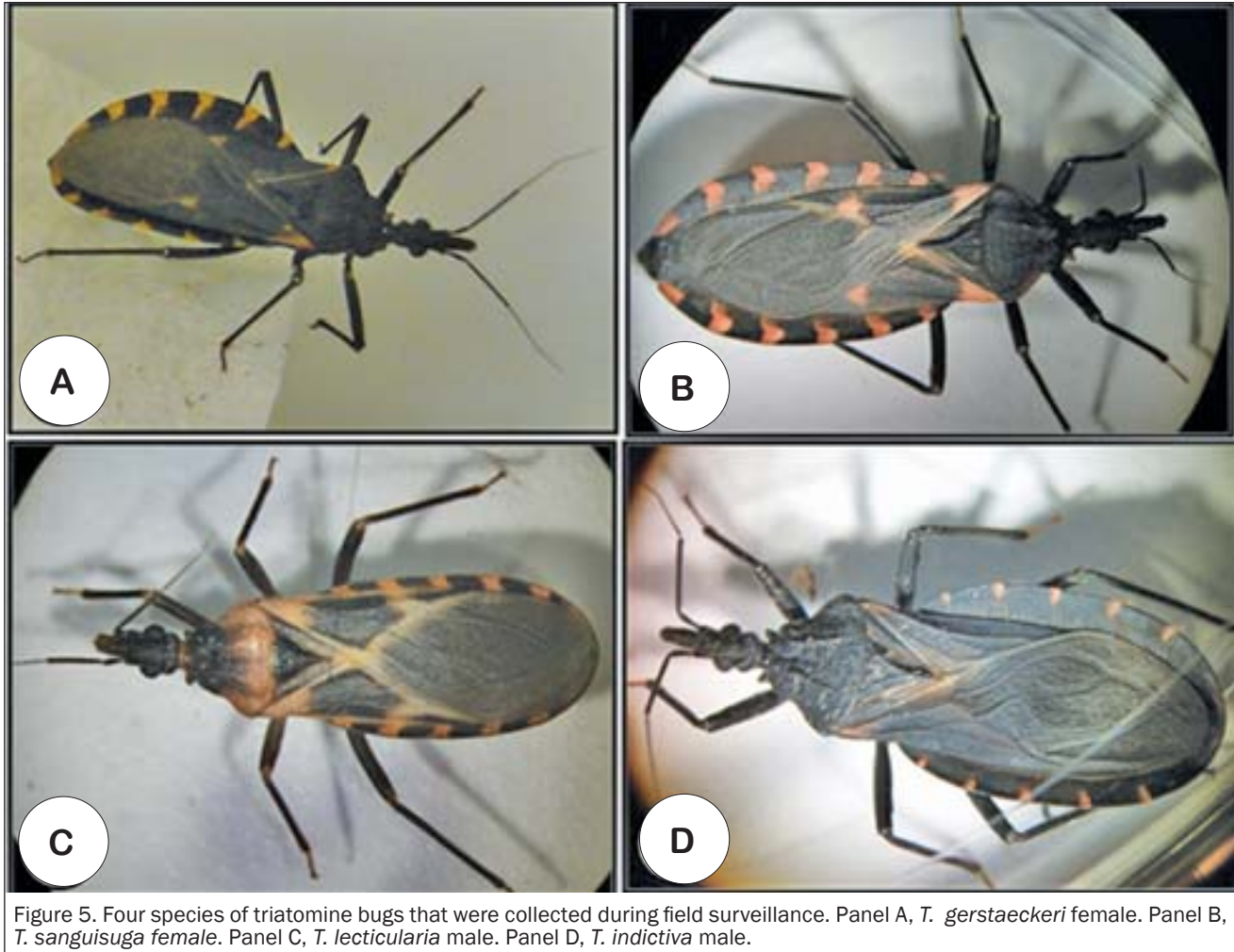


Figure 5. Four species of triatomine bugs that were collected during field surveillance. Panel A, *T. gerstaeckeri* female. Panel B, *T. sanguisuga* female. Panel C, *T. lecticularia* male. Panel D, *T. indictiva* male.

transmission (*T. infestans* (Klug)) was more urban and cohabitated with humans. North American species are more elusive and mainly sylvatic. The 2 most common techniques cited in the literature are light trapping and wood rat nest excavation. These two collection methods rarely give a reliable indication of population size.

The wood excavation method was the most effective surveillance technique in this study because we collected all life stages for 4 different *Triatoma* species. During the peak of the summer, this method proved to be consistently effective for collecting large numbers of specimens. However, this method is labor intensive and requires skill for habitat acquisition.

Possible oral transmission of Chagas disease poses a challenge for the control of Chagas vectors at the MWD Center. Conventional pesticide applications would not be effective due to the cleaning regimen and could possibly expose the dogs to dead or dying insects. Control of the reservoir hosts (rodents) in the woodland habitat

with rodenticides could lead to secondary poisoning of the military working dogs.^{24,25}

A solution to the problem would be the use of a systemic insecticide for the control of the triatomine vectors. Systemic insecticides attack insects directly through their living food sources without harming the host species, and can be impregnated into a grain bait to treat the rodents and any other potential reservoir hosts in the wooded areas around the MWD Center. Pesticides with low mammalian toxicity are available, and should pose no unreasonable threat to the military working dogs.²⁶ Kaput Rodent Flea Control Bait (EPA Reg. No. 72500-17) is an imidicloprid systemic bait well suited for this application. This product can be used in accordance with the Federal Insecticide, Fungicide, and Rodenticide Act,²⁷ Section 2ee, by treating the site specifically as described on the product label.

Field collected specimens were mainly collected in habitats that included hollow logs of different species (live

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oak and cedar) and dead dry yucca plants. Triatomines were rarely found in rodent nests. The most common habitat conditions included a hollow log over or near an active rodent burrow. It appeared that the kissing bugs entered the nest to feed, and then retreated to harbor-age for protection. Various arthropods are often found cohabitating inside the logs with the kissing bugs (scorpions, wood boring beetles, pill bugs, and centipedes). Acorns, rodent droppings, and nesting material were also indicators for positive habitat association. If fire ants were present near or in the log or burrow, no kissing bugs were found.

In summary, Chagas disease continues to pose significant health risks to the military working dog population in San Antonio. In order to develop an effective control method, further research is warranted to understand both biology and ecology of local triatomine species.

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